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## EUROPEAN PATENT APPLICATION

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PHARMACEUTICAL  
PHARMA COMPNSN. CONTG. CALCITONIN AND SURFACTANT  
+ FOR PROLONGED INTRANASAL ADMIN. WITH BONE  
METABOLISM DISORDERS

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㉒ Enhancement of intranasal absorption of calcitonin by formulation with surfactants.

㉓ A pharmaceutical composition for the treatment of disorders of bone metabolism which comprises an aqueous or non-aqueous medium suitable for intranasal administration and containing a therapeutically effective amount of calcitonin and a surface active agent.

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1 ENHANCEMENT OF INTRANASAL ABSORPTION OF  
CALCITONIN BY FORMULATION WITH SURFACTANTS

5 The present invention relates to a novel method of  
administering calcitonin to patients and to formulations  
adapted for nasal administration.

10 Calcitonin is a polypeptide hormone isolated from  
different organs in different species, including man and  
salmon, or obtained via synthetic routes. Calcitonin is  
recognized as being effective in diminishing hypercalcemia  
and decreasing plasma phosphate concentrations in patients  
with hyperparathyroidism, idiopathic hypercalcemia of  
infancy, vitamin D intoxication, and osteolytic bone  
metastases. While direct renal effects and actions on the  
gastrointestinal tract are recognized, calcitonin is best  
15 known for its effect on bone. Its use has proved to be  
effective in diseases characterized by increased skeletal  
resorption and abnormal bone formation, such as occurs for  
example, in Paget's disease.

20 The method of administration of calcitonin is  
predominantly by injection, although efforts were made in the  
prior art to use other modes of administration, especially  
for the treatment of localized conditions. While injectable  
administration by physicians of calcitonin is proper for  
short-term therapy, administration of calcitonin by injection  
25 to patients in need of long-term calcitonin therapy has a  
serious problem. Not only is it costly to patients to have  
physicians do the administration of calcitonin for extended  
periods of time but it is also painful and inconvenient. Nor

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1 can calcitonin be given orally to patients as it will be destroyed by the digestive juices in the gastrointestinal tract.

5 In view of the foregoing, it is apparent that a serious need exists for a different route of delivery of calcitonin to patients suffering from conditions that require prolonged calcitonin therapy.

0 Nasal preparations are known in the prior art. Generally, nasal preparations comprise an oil-in-water or water-in-oil emulsion or an oily solvent base suitable for use on the mucous membranes, such as mineral or vegetable oils and fatty acid esters and one or more chemicals which are soluble in the base. Such preparations usually contain one or more active drugs intended to alleviate or mitigate a condition in the body by their adsorption into the blood stream through the mucous membrane of the nose.

) While small molecules such as propranolol are efficiently absorbed intranasally, large molecules such as calcitonin show little if any absorption. The purpose of this invention is to find agents capable of increasing the bioavailability of calcitonin so that cost of therapy is reasonable. The prior art has also recognized that the nasal absorption of certain drugs may be facilitated by the use of surfactants in such nasal preparation. For example, insulin and polypeptides were found to have an improved absorption rate when used in a solution containing a surfactant.

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1 It has now been found that hypercalcemia, Paget's  
disease and other disorders of bone metabolism can be  
advantageously treated by intranasal application of  
calcitonin contain in a nasal preparation having an  
5 absorption promoter and a buffer as essential ingredients.  
Such preparations possess enhanced absorption across the  
nasal mucosa when applied intranasally, but causes no  
irritation or discomfort on extended use.

10 The present invention relates to a method for the  
treatment of a mammal suffering from a disorder characterized  
by high serum calcium which comprises intranasal application  
of a nasal preparation containing a peptide having calcitonin  
activity and an absorption promoting agent to effect control  
of said disorders by transepithelial action.

15 According to the invention, calcitonin is  
intranasally administered to a mammal via a novel dosage  
form, such as a solution, ointment, or gel.

20 Calcitonin is a peptide hormone of 32 amino acids  
with a disulfide bond at 1-7 in the amino terminus of the  
molecule. These first seven amino acids with the disulfide  
bond seem essential for activity and this sequence is  
preserved from species to species. Calcitonin, as used  
herein, means not only peptides having a structure  
corresponding to one of the naturally occurring hormones, and  
25 which may be naturally or synthetically produced, but also  
related peptides having calcitonin activity.

The amount of calcitonin contained in the  
preparation of the present invention may vary according to  
various parameters, such as the nature of the preparation,

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1 the particular kind or activity of calcitonin employed and  
the condition or ailment to be treated with the preparations.  
In general, the concentrations are somewhat higher than those  
found in compositions for the systemic administration of  
5 calcitonin. It has been found that a concentration level of  
1 to 150 micrograms per ml and preferably 2 to 30 micrograms  
per ml achieve the desired result. The levels of  
administration of calcitonin also vary somewhat from those  
used systemically. In the case of human patients, for  
10 example, amounts of from 0.7 to 70 micrograms, particularly  
from 1 to 25 micrograms, are usually appropriate for single  
dosages given and repeated as often as the physician finds it  
necessary and such dosages correspond generally to about 0.01  
to 1 micrograms, and particularly 0.03 to 0.35 micrograms,  
15 per kilogram of body weight. (The above concentration and  
dosage levels of calcitonin apply to calcitonin with a  
potency of about 4000 International Units per mg and may be  
adjusted pro rata for calcitonin of other potencies.)

The diluent base or vehicle used in accordance with  
20 the present invention may be non-aqueous or aqueous. In the  
former case the group of diluents is the physiologically  
acceptable polar solvents. Preferred compounds of this type  
are those with which it is possible to make a solution of  
adequate concentration of dissolved calcitonin. Examples of  
25 these compounds include dimethylsulphoxide, dimethyl  
formamide, dimethyllauramide, polyhydroxy alcohols, vegetable  
and mineral oils. If desired, such non-aqueous media may be  
mixed with water to form the diluent of the preparation.

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1 However, the degree of physiological acceptability of the  
non-aqueous diluents is generally less than that of aqueous  
media and the preferred diluent is therefore water without  
the addition of organic solvents.

5 In the preparations of the present invention,  
calcitonin is used in combination with an absorption  
promoter. Such absorption promoters include the  
physiologically acceptable surface active agents. The amount  
of such an agent may be in the range from about 0.01 to about  
10 10% w/v or higher and preferably about 0.05 to about 1.0%  
w/v, the amount depending on the specific surfactant used.  
The amount is generally kept as low as possible since above a  
certain level no further enhancement of absorption can be  
achieved and also too high of a surfactant level may cause  
irritation of the nasal mucosa. Such surface active agents  
15 include:

- a. Bile salts, such as sodium taurocholate, sodium cholate,  
sodium deoxycholate and sodium glycholate;
- b. Cationics, such as the long chain amine condensates with  
ethylene oxide and quaternary ammonium compounds, for example  
20 cetyl trimethyl ammonium bromide and dodecyl dimethyl  
ammonium bromide;
- c. Anionics, such as alkylbenzenesulfonates,  
N-acyl-n-alkyltaurates,  $\alpha$  -olefin sulfonates, sulfated  
25 linear primary alcohols and sulfated polyoxyethylenated  
straight-chain alcohols;
- d. Nonionics, such as polyoxyethylenated alkylphenols,  
polyoxyethylenated straight chain alcohols, long chain  
carboxylic acid esters including glycerol ester of natural  
30 fatty acids, propylene glycol, sorbitol, and  
polyoxyethylenated sorbitol esters;

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- 1 e. Amphoteric, such as imidazoline carboxyslates,  
sulfonates and the like; and  
f. Phospholipids, such as phosphotidyl choline and the like.

5 The preparations of the present invention  
preferably contain a phosphate or acetate buffer in the range  
of 0.01 M to 0.5 M and preferably in the range of 0.05 M to  
0.2 M. This concentration was found effective to provide  
stability of the dissolved calcitonin in the diluent base or  
vehicle.

10 The preparations of the present invention may also  
contain other additives, such antioxidants, stabilizers,  
tonicity adjusters, viscosity builders, preservatives, and  
the like. The concentration of these additives may vary  
according to the particular additive used and the desired  
result sought. In general, the concentrations for these  
15 additives will be in the range as follows:

<u>Additives</u>	<u>% W/V</u>
Antioxidants	0.01 - 0.2
Stabilizers	0.01 - 2.0
Tonicity Adjuster	0.01 - 0.5
Viscosity Builders	0.1 - 2.0
Preservatives	0.001 - 2.0

25 While the use of the kind and concentration of  
additives will be well within the ability of the skilled  
artisan, the following will serve as illustration for two  
additives generally used in pharmaceutical preparations  
intended for similar purposes.

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	<u>Preservatives</u>	% W/V
1	Benzalkonium chloride	0.004 - 0.02
	Disodium Ethylene	
	Diamine Tetraacetate	0.01 - 0.2
5	Thimerosal	0.001 - 0.01
	Chlorobutanol	0.5 - 1.0
	Methyl and/or Propyl Paraben	0.01 - 0.2
	Phenethyl Alcohol	0.25 - 0.75
10	Cyclohexidine	0.01 - 0.1
	<u>Viscosity Agents</u>	% W/V
	Methyl Cellulose	0.1 - 2.0
	Hydroxyethyl Cellulose	0.1 - 2.0
15	Hydroxypropyl Cellulose	0.1 - 2.0
	Polyvinylprrolidone	0.5 - 2.0

20 In preparing the formulations of the present invention, calcitonin is dissolved in the vehicle or diluent after which the additional ingredients are added in accordance with customary formulation procedures known in the pharmaceutical industry.

25 Examples of typical intranasal formulations are set forth below. However, it is to be understood that these examples are given by way of illustration only and are not to be construed as limiting the invention either in spirit or in scope as many modifications will be apparent to those skilled in the art.  
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EXAMPLE 1                    % W/V

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Calcitonin	0.009
Sodium Taurocholate	0.5
Gelatin	1.0
Purified Water Q.S.	100

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EXAMPLE 2                    % W/V

15

Calcitonin	0.009
Miranol C2M	1.0
Gelatin	1.0
Purified Water Q.S.	100

20

EXAMPLE 3                    % W/V

Calcitonin	0.009
Miranol C2M	0.05
Sodium Acetate .3H <sub>2</sub> O	1.36
Acetic Acid	0.6
Purified Water Q.S.	100

25

EXAMPLE 4                    % W/V

30

Calcitonin	0.009
Polysorbate 80	1.0
Sodium Acetate .3H <sub>2</sub> O	1.36
Acetic Acid	0.6
Purified Water Q.S.	100

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EXAMPLE 5                  % W/V

Calcitonin	0.003
Brij 30	1.0
Sodium Acetate .3H <sub>2</sub> O	1.36
Acetic Acid	0.6
Purified Water Q.S.	100

EXAMPLE 6                  % W/V

Calcitonin	0.009
Myrj 59	1.0
Sodium Acetate	1.36
Acetic Acid	0.6
Purified Water Q.S.	100

EXAMPLE 7                  % W/V

Calcitonin	0.009
Miranol C2M	1.0
Sodium Phosphate	2.40
Citric Acid	0.34
Thimerasol	0.002
Purified Water Q.W.	100

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<u>EXAMPLE 8</u>	<u>% W/V</u>
Calcitonin	0.009
Sodium Taurocholate	0.5
Sodium Acetate .3H <sub>2</sub> O	1.36
Acetic Acid	0.6
Benzalkonium Chloride	0.01
DiSodium ethylenediamine tetraacetate	0.1
Purified Water Q.S.	100

<u>EXAMPLE 9</u>	<u>% W/V</u>
Calcitonin	0.009
Sodium Taurocholate	0.5
Sodium Acetate .3H <sub>2</sub> O	1.36
Acetic Acid	1.36
Chlorobutanol	0.1
Phenethyl Alcohol	0.2
Purified Water Q.S.	100

<u>EXAMPLE 10</u>	<u>% W/V</u>
Calcitonin	0.003
Miranol C2M	1.0
Sodium Phosphate	2.40
Citric Acid	0.34
Thimerasol	0.002
Purified Water Q.S.	100

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The gelatin used in the above formulations is a standard hydrolyzed animal gelatin prepared for pharmaceutical use and routinely used as a diluent for peptides.

According to the present invention, it has been found that calcitonin can be administered intranasally from a vehicle containing absorption promoters with results considerably superior to those obtained with the administration of calcitonin without absorption promoters. The following studies were undertaken to examine the bioavailability of calcitonin from the formulations of the present invention, dependency of intranasal absorption of calcitonin on the level of absorption promoters and stability of calcitonin in the presence of absorption promoters.

PROTOCOL

Male rats weighing 150-250 g were weighed and anesthetized with sodium pentobarbital, 50/mg/kg. by intraperitoneal injection. Once anesthetized the nasopalatine process was occluded with glue. The animals were randomly placed into groups of 5-7 rats with the number of groups being dependent upon the number of intranasal formulations to be tested. Supplemental pentobarbital anesthesia was administered as necessary throughout the study.

Prior to administration of the test material, blood was collected by cardiac puncture using a 25G 5/8" needle. Fifty (50) microliters of the salmon calcitonin-containing surfactant solution was then instilled into the nasal septum using polyethylene tubing (PE 20, Peterson Technics, Monmouth Junction, N.J.) connected to a 1 ml syringe; the tubing was inserted about 1 cm into the nasal septum. One and three

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1 hours after nasal instillation, blood was again collected by  
cardiac puncture.

Biochemical Analysis

5 Blood samples were allowed to clot at room temperature and were then refrigerated for 30-60 minutes to provide maximum clot-retraction. The samples were centrifuged at 4°C., 5000 rpm for 10 minutes (Beckman Model J2-21 Centrifuge, Beckman Instruments, Palo Alto, CA). Serum calcium was quantitated using a Calcette (Model 4008, 10 Precision Systems, Sudbury, MA).

Data Analysis

15 Serum calcium values at 0, 1 and 3 hours were expressed as mean  $\pm$  standard deviation. In addition, the absolute change and the percent change from the pretreatment (0 time) value at 1 and 3 hours was also calculated. Statistical analysis consisted of comparison of the serum calcium values at 0 and 1 hour, 0 and 3 hours, and 1 and 3 hours using a t test.

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EXAMPLE 11

This example illustrates decrease in serum calcium in blood samples obtained in accordance with the above protocol when: a. calcitonin is administered alone; b. calcitonin is administered in formulations containing various absorption promoters; and c. no calcitonin is present in the formulations.

Table 1 shows the result obtained.

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TABLE I

Calicitonin U/kg body weight vehicle/surfactant	0 hour mg/dl	TIME AFTER DOSE		
		1 hour mg/dl.	% decr.	3 hour mg/dl. % decrease
2U 1% gel -	8.8	9.5	NONE	9.9
5U 1% gel -	8.5	7.9	7.1	9.5
5U 1% gel -	9.2	7.6	17.4	9.7
7U 10U .1M Acetate -	8.8	6.3	28.4	9.0
- 1% gel 1% Miranol C2N (1)	8.9	8.8	NONE	9.2
- 1% gel 1% Taurocholate	9.1	9.5	NONE	9.8
3U 1% gel 1% Miranol C2N (1)	8.9	6.7	24.7	7.6
8.8	6.5	26.1	8.5	3.4
8.7	6.8	21.8	8.9	2.3
3U 0.1M Acet. 1% Miranol C2N (1)	8.7	6.8	21.8	8.9
10U 1% gel 1% Miranol C2N (1)	9.5	6.1	35.8	8.8
9.1	7.5	17.6	6.6	27.5
8.9	7.1	20.2	7.1	20.2
10U 0.1M Acet. 1% Miranol C2N (1)	9.3	6.1	34.4	6.5
9.2	6.8	26.1	8.4	8.7
3U 1% gel 1% Taurocholate	9.1	7.0	23.1	6.4
10U 1% gel 1% Taurocholate	9.3	7.1	23.7	6.3
8.6	6.1	29.1	5.9	31.4
10U 0.1M Acet. 1% Taurocholate	9.1	6.5	28.6	5.7
8.3	6.2	25.3	8.7	NONE
3U 1% gel 1% Tween 80 (Polysorbate 80)	8.9	7.1	20.2	9.2
				NONE

(1) Dienthydroxylated fatty imidazoline or dicarboxylic coconut derivative,

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TABLE I (Cont'd.)

Calcitonin U/kg body weight vehicle/surfactant	0 hour mg/dl	TIME AFTER DOSE			<u>mg/dl</u> <u>% decrease</u>
		1 hour mg/dl	% decr.	3 hour mg/dl	
10U 1% gel 1% Tween 80 (Polysorbate)	8.7	6.6	24.2	7.3	16.1
3U 1% gel 0.5% Benzal- konium Chloride	8.9	6.7	24.7	8.4	5.6
	8.7	5.9	32.2	6.3	27.6
10U 1% gel 0.5% Benzal- konium Chloride	8.9	6.5	26.9	9.0	NONE
	8.5	7.2	15.3	7.9	7.1
3U 1% gel 1% Saponin (Sapogenin Glycoside)	9.0	6.0	33.3	6.2	31.0
	8.6	7.3	15.1	7.3	15.1
10U .1M Acet. 1% NaL Saf	8.7	6.5	25.9	8.1	6.9
10U .1N Acet. 1% Brij 30	8.7	6.5	25.9	6.5	25.9
Polyoxyethylene (4) lauryl ether)					
10U .1N Acet. 1% Myrj 59	8.5	6.2	27.1	8.4	1.2
(Polyoxyethylene (100) Stearate)					
10U .1M Acet. 1% Tween 80	8.7	7.5	13.8	7.1	18.4
10U .1N Acet. 1% Aer OT	9.1	6.6	27.5	7.6	16.5
Sodium dioctyl sulfosuccinate)					

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EXAMPLE 12

This example illustrates that the enhancement of intranasal absorption depends on the level of absorption promoter present in the formulation.

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Table II shows the result obtained.

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TABLE II

<u>10U Calcitonin/kilo in 0.1M Acetate with</u>	<u>0 hour mg/dl</u>	<u>1 hour mg/dl</u>	<u>3 hour %</u>
1% Taurocholate	9.1	6.5	28.6
0.5%	9.0	6.1	32.2
0.25%	9.1	6.8	25.3
0.1%	8.9	6.5	27.0
0.05%	9.0	7.6	15.5

10U calcitonin/kilo  
in 0.1M Acetate with

<u>1% Miranol C2N (dicarboxylic coconut derivative, sodium salt)</u>
0.5%
0.25%
0.1%
0.5%

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EXAMPLE 13

This example illustrates that calcitonin maintains its activity level in the formulations of the present invention on storage at room temperatures.

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Table III shows the results obtained.

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TABLE III

58 10U calcitonin in 1% gel		0 hour		1 hour		$\frac{mg/dl}{mg/dl}$ hour	
Initial		8.9	7.1	20.2	7.1	20.2	
2 wks @ RT		9.1	7.2	20.9	6.4	29.7	
4 wks @ RT		9.1	6.0	34.1	6.9	24.2	
10U calcitonin in 1% gel with 1% Tween 80 (Polysorbate 80)							
Initial		8.9	6.7	24.7	8.4	5.6	
2 wks @ RT		8.8	6.7	23.9	8.3	6.0	
4 wks @ RT		8.8	6.3	28.4	6.7	23.9	

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DOCUMENTS CONSIDERED TO BE RELEVANT			EP 83113070.3
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 7)
A	<p><u>US - A - 4 241 051</u> (CHRISTIE et al.)</p> <p>* Claims 3,4,5,7-10; column 1, line 45 - column 4, line 46-*</p> <p>--</p>	1,6	A 61 K 37/02
A	<p><u>GB - A - 1 548 984</u> (CIBA-GEIGY AG)</p> <p>* Claims, especially claim 5; page 1, line 9 - page 3, line 66 *</p> <p>--</p>	1,2,5	
A	<p><u>DE - A1 - 2 254 061</u> (HOECHST AG)</p> <p>* Claim 1,4; pages 1,2 *</p> <p>-----</p>		
			TECHNICAL FIELDS SEARCHED (Int. Cl. 7)
			A 61 K 37/00
584			
The present search report has been drawn up for all claims			
Place of search	Date of completion of the search	Examiner	
VIENNA	30-03-1984	STÖCKLMAYER	
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